

amino acid sequence motif, ⁷⁵LIXXGVXXG⁸³VXXT, which is proposed to be essential for dimerization in glycoprotein A is similarly observed on TMVI of β_2 AR as in LKTLG²⁷⁶IMMG²⁸⁰TFTL²⁸⁴. A peptide is derived from TMVI consisting of residues from 276 to 296, GIIMGTFTLCWLPFFIVNIVH and blindly docked to one conformer of the monomeric structure using a rigid body approach, via AutoDock v4.0 software tool [3]. Bound conformations were then reevaluated with a knowledge-based scoring function called DSX^{online} v0.88 [4]. Docking results shows that the peptide has the highest binding affinity for TMVI which supports the possible role of TMVI at the interface of a dimeric structure. Also, a residue interface propensity data derived from a set of 32 nonhomologous homodimers [5] is able to identify TMVI as the domain which contains the highest number of residues with the highest interface propensity values.

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2387-Pos Board B157

Characterizing the Motion of W6.48 in the Active State of a GPCR

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Early biochemical and biophysical studies of GPCRs (G-Protein Coupled Receptors) have suggested that there are a series of switches which trigger the conformational changes associated with activation[1]. These switches include i) the rupture of the cytosolic "ionic-lock" between the E/DRY motif on TMH3 and the intracellular end of TMH6, with a subsequent outward displacement of TMH6, as well as ii) a rotameric transition of W6.48, a modulator of the geometry of TMH6 about the conserved P6.50. This "toggle switch" mechanism effectively couples ligand binding to intracellular conformational changes necessary for activation. Recently several activated state crystal structures have been released, of particular significance is the agonist bound β_2 AR in complex with the Gs heterotrimer[2]. This state is noteworthy as it is the first structure of an activated GPCR in complex with its cognate G-protein. Although these structures display the expected outward movement of TMH6, none indicate a rotameric transition of W6.48, implying that a rotameric change is not required for activation. Alternately, it has been suggested that the transition is transitory[3]. Given that the β_2 AR displays constitutive activity[4], it seems plausible that the energy required to transition from the inactive to the active state is thermally accessible. In order to test the hypothesis that W6.48 makes transient rotameric changes we have undertaken microsecond long molecular dynamics simulations embedding the full β_2 AR complex, derived from the crystal structure, into an all atom phospholipid environment. The thermal motion/fluctuations available to the complex and the propensity of W6.48 to undergo rotameric changes will be discussed in light of recent structural studies.

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2388-Pos Board B158

Molecular Determinants for a Mutant Mu-Opioid Receptor in which Naloxone Acts as a Partial Agonist

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The Law group has identified a mutant μ -opioid receptor (MOR), S4.54A, at which traditional opioid alkaloid antagonists become agonists (Yang et al., 2003). Subsequent targeted gene therapy studies using the S4.54A mutant receptor have indicated that naloxone can act as an antinociceptive agent *in vivo* (Chen et al., 2007). The goal of the work presented here was to understand at the molecular level, the origins of this unusual phenotype. Compounding the unusual nature of this mutant is the fact that S4.54 is a lipid facing residue. Ballesteros and colleagues have reported that the hydrogen bonding capacity of lipid facing Ser/Thr residues in α -helices can be satisfied by an intrahelical hydrogen bond interaction, in either the *g*- or *g*+ conformation, between the O- γ atom and the *i*-3 or *i*-4 carbonyl oxygen (Ballesteros et al., 2000). Ser/Thr residues in the *g*- conformation can induce a bend in an α -helix, and we have found that changes in wobble angle and face shift can also occur (Ballesteros et al.,

2000). To explore the effect of the S4.54A mutation on the conformation of TMH4, we used the Monte Carlo/simulated annealing technique, Conformational Memories (CM) (Konvicka et al., 1998; Whitnell et al., 2008). CM results showed that the S4.54A mutation alters the conformation of TMH4, such that the top of TMH4 bends into the bundle towards TMH3. When the output S4.54A mutant TMH4 was incorporated into our inactive state MOR model, packing of the TMHs in the TMH3/TMH4 region was altered such that the opioid antagonist, naloxone could no longer stabilize the toggle switch residue, W6.48 in its inactive state conformation. [Support: DA023905, P-Y L; DA021358, PHR]

2389-Pos Board B159

TMBB-Explorer: A Webserver to Predict the Structure, Oligomerization State, Ppi Interface, and Thermodynamic Properties of the Transmembrane Domains of Outer Membrane Proteins

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Beta-barrel membrane proteins are found in the outer membrane of gram-negative bacteria, mitochondria, and chloroplasts. They are important for pore formation, membrane anchoring, enzyme activity, and are often responsible for bacterial virulence. Due to difficulties in experimental structure determination, they are sparsely represented in the protein structure databank. We have developed a webserver (TMBB-Explorer) capable of predicting the three-dimensional structure, oligomerization state, protein-protein interaction interface, and thermodynamic properties such as heat capacity and relative melting temperature of the transmembrane domains of beta-barrel membrane proteins. Our method is based on a physical interaction model, a simplified conformational space for efficient enumeration, and an empirical potential function from a detailed combinatorial analysis [1]. It also accounts for loop entropy and an affinity for right-handedness of the natural beta-barrels. The webserver also reports the hydrogen bonds formed between the beta-strands in the predicted structure. The webserver can provide predictions using sequence information alone or by incorporating secondary structure information. [1] Naveed et al. *Proc. Natl. Acad. Sci. USA*, 106(31):12735-12740.

2390-Pos Board B160

Memprotmd: Adding the Grease to Membrane Protein Structures

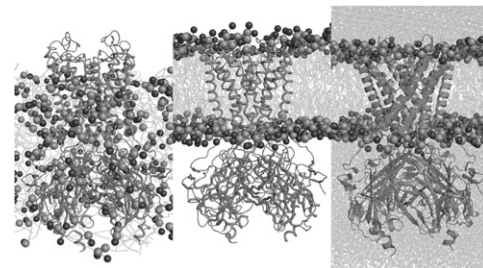
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Membrane protein structural biology is one of the key biochemical challenges. With continuous improvements to the methods used by structural biologists there is a predicted exponential growth in the number of membrane proteins structures. Nevertheless, these biological assemblies are usually resolved in the absence of the native lipid environment. Coarse-Grained molecular dynamics (CGMD) simulations provide a means for assessing the assembly and interactions of molecular complexes at a reduced level of representation. This method has been shown to accurately predict the position and orientation of proteins within a cell membrane. The results of these predictions are available in a database (<http://sbcb.bioch.ox.ac.uk/cgdb>). We are in the process of pipelining the procedure, so that new membrane protein structures are automatically inserted into a DPPC lipid bilayer on release from the Protein Data Bank (PDB). The CG simulations are then assessed for protein-lipid interactions, bilayer deformation, lipid diffusion and protein tilt. The resulting models are then refined to include more physiologically relevant lipid mixtures and subsequently converted to an atomistic resolution [1] to enable more detailed simulations of lipid protein interactions [2].

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2391-Pos Board B161

Identification and Characterization of Transmembrane Segments

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Membrane proteins comprise 30-40% of all known proteins; however, they remain the most uncharacterized. The protein data bank contains very few detailed 3D structures of the transmembrane segments of transmembrane

proteins. This project seeks to determine which amino acids are necessary or present in the transmembrane segment as well as the juxtamembrane segments. It also seeks to better characterize the physical orientation of the fibroblast growth factor receptor transmembrane segment in the membrane and how the amino acids are oriented within the helix. An exclusive database of transmembrane proteins and juxtamembrane domains was created to search for trends, homology, and potential phosphorylation sites. Even though only a very limited amount of homology was found, the transmembrane segment from the fibroblast growth factor receptor will be used as a model and synthesized and characterized through a variety of biophysical techniques such as multidimensional NMR spectroscopy, circular dichroism, and fluorescence spectroscopy.

2392-Pos Board B162

An Empirical Scoring Function for the Transmembrane Helical Protein Assembly

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We present a coarse-grained scoring energy able to identify near-native transmembrane (TM) helices pairs of an ensemble generated by two different strategies: Rigid Body Monte Carlo simulations and a collision detection algorithm (Seetharaman et al., work in progress). We test the scoring energy on a set of four known native transmembrane helix pairs. Near native structures are identified with C_α root mean squared deviation (RMSD) lower than 3 Å. This empirical energy function is based on a knowledge based potential obtained from a representative set of globular protein structures. We compensate packing differences of globular and helical membrane protein structures by adding a residue solvent accessible area energy term based on a membrane partition scale obtained from the membrane insertion by the Sec translocon (Hessa et al., Nature 450, 1026-1030, 2007). In addition, we strengthen the interactions among small and polar amino acids that improve the scoring of topologically correct near-native structures. We conclude that our scoring energy function favors near-native conformations of TM dimers without structural knowledge extracted from the yet small set of known membrane protein structures. This proposed method circumvents intensive membrane protein molecular dynamics simulations opening the possibility of further refinement of near-native TM structures through atomistic MD simulations.

2393-Pos Board B163

Structure-Functional Insight into Transmembrane Helix Dimerization by Protein Engineering, Molecular Modeling and Heteronuclear NMR Spectroscopy

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The interaction between transmembrane helices is of a great interest because it directly determines biological activity of membrane proteins. Either destroying or enhancing such interactions can result in many diseases related to dysfunction of different tissues in human body. One of the most common forms of membrane proteins is a dimer containing two membrane-spanning helices associating laterally to form a tight complex. Development of new types of drugs targeting membrane proteins requires precise structural information about this class of objects. Recent development of protein engineering, optical spectroscopy, molecular modeling and heteronuclear NMR techniques made it possible studies of the nature and mechanisms of important helix-helix interactions inside the membrane mimicking supramolecular complexes. Using a robust strategy we investigated recombinant transmembrane fragments from different families of bitopic membrane proteins including receptor tyrosine kinases, amyloid precursor and pro-apoptotic proteins, which play important roles in normal and pathological conditions of human organism by providing cell signaling, maintaining cellular homeostasis and controlling cell fate. We characterized thermodynamics of transmembrane helix association, diverse helix-helix packing interfaces and obtained detailed atomistic picture of the intra- and intermolecular (protein-protein, protein-lipid and protein-water) interactions, that along with the available biochemical data provided useful insights into the membrane protein functioning in normal and pathology.

This work was supported by RFBR, the Program of RAS "MCB", the Federal Target Programs "Scientific and Pedagogical Specialists of Innovation Russia (2009-2013)" and "Research and development in priority fields of Russian scientific and technological complex in 2007-2012".

2394-Pos Board B164

Transmembrane Helix-Helix Interactions in the Human Single-Span Membrane Proteome

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Most integral membrane proteins form non-covalent functional complexes that are frequently supported by sequence-specific interaction of transmembrane helices [1]. It has been suggested that non-covalent membrane protein multimerization may substitute for the frequently observed multi-domain organization of soluble proteins [2,3]. Here, we aligned human single-span membrane proteins with orthologs from other eukaryotes and examined the sidedness of transmembrane helices. We find that almost half of the human single-span membrane proteins possess a transmembrane helix with unilateral conservation. We propose unilateral conservation in most cases to indicate the presence of a helix-helix interface as well as the strength of interaction since it correlates well with experimentally determined self-affinities. This suggests that unilateral conservation is a good predictor of homotypic TMD interaction and underlines that transmembrane helix-helix interactions significantly contribute to protein assembly in the human single-span membrane proteome.

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2395-Pos Board B165

Self-Association of Transmembrane Domains of ErbB2 Receptors in Cholesterol-Containing Membranes

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The transmembrane domain of ErbB2 receptors presents two separate GxxxG motifs that are proposed to be connected to stability and activity of the dimer. Recently developed parallel Monte Carlo methods are employed to study the association of ErbB2 TM domains in cholesterol-containing membranes with coarse-grained models that retain a level of amino-acid specificity. Extensive sampling along separation between the two helices shows that GxxxG motifs play a critical role during the recognition stage. In pure phospholipid bilayers association occurs by contacts formed at the C-terminus promoted by the presence of phenylalanine residues. Helices subsequently rotate to eventually form a stable dimer favored by lipid entropic contributions. In contrast, at intermediate cholesterol concentrations a different pathway is followed that involves dimers with a weaker interface towards the N-terminus. However, at high cholesterol content, a switch towards the C-terminus is observed with an overall non-monotonic change of the dimerization affinity. This conformational switch modulated by cholesterol has important implications on the thermodynamic, structural and kinetic characteristics of helix-helix association in lipid membranes.

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2396-Pos Board B166

Assembling the Transmembrane Domain of Vpu from HIV-1

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Vpu from HIV-1 is an 81 amino acid monotopic viral membrane protein involved in the amplification of viral replication. Vpu is identified to down regulate membrane proteins of the host e.g. CD4, CD74, CD317 and BST-2/Tetherin. Based on the findings that Vpu exhibits channel activity especially when reconstituted into lipid membranes the protein is also proposed to act as a viral channel forming protein (VCP) in vivo. How Vpu is supposed to form the channel is unknown.